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Fungal Biodegradation of Procyanidin in Submerged Fermentation

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Abstract: The coffee industry generates a large amount of waste that is difficult to treat due to its chemical composition, namely, the presence of caffeine and its derivatives, as well as recalcitrant molecules such as tannins (mainly condensed tannins or polymeric procyanidins), which make it an undervalued waste product. Procyanidins are compounds beneficial to human health and can be found in nature in fruit, grain, seeds, and beverages, among other foods. The zero-waste approach has allowed for the valorization of by-products from the food industry. Currently, coffee pulp is the target of research on extraction, purification, and alternative use. Research on the fungal degradation of procyanidins has emerged as an avenue for the efficient use of these by-products. In this study, the degradation and biotransformation of procyanidin is evaluated and comprises three steps: first, the extraction and partial purification of procyanidins from coffee pulp; second, the production of the potential procyanidin-degrading enzyme by submerged fermentation with *Aspergillus niger* GH1; third, enzymatic extracellular extract evaluation using a model system with commercial procyanidin C1. The biodegradation/biotransformation results reveal the formation of new compounds, including a final compound with an m/z of 289, possibly a monomeric molecule such as catechin or epicatechin. Identification of the compounds by HPLC-MS confirmed procyanidin C1 depletion under the described assay conditions, which could be used to understand biodegradation pathways proposed for future study. Furthermore, these results confirm that *A. niger* GH1 is able to degrade and biotransform procyanidin C1.

Keywords: procyanidin; biodegradation; submerged fermentation; extracellular enzyme; *Aspergillus niger* GH1

Academic Editors: Giovanni Vigliotta and Rosanna Tofalo

Received: 27 December 2024
Revised: 23 January 2025
Accepted: 29 January 2025
Published: 3 February 2025

Citation: Valencia-Hernández, L.J.; Wong-Paz, J.E.; Ascacio-Valdés, J.A.; Prado-Barragan, A.; Chávez-González, M.L.; Aguilar, C.N. Fungal Biodegradation of Procyanidin in Submerged Fermentation. *Fermentation* **2025**, *11*, 75. https://doi.org/10.3390/fermentation11020075

1. Introduction

Procyanidins (PCs) are macromolecules formed from the condensation of catechin or epicatechin monomers [1]. Today, the biological potential of these macromolecules has enabled remarkable advances in the medical, biological and phytochemical fields [2]. The commercialization of these compounds is limited due to costly and tedious extraction and purification processes [3]. However, PCs are commercially available as purified standards and as dietary supplements in the form of procyanidin oligomers (PCOs). Plant sources, such as grape seed, pine bark, blueberry, litchi peel, and apple, among others, have been reported for their potential use in extraction and purification methodologies [4].

Currently, some research groups are interested in the use of cheap agro-industrial waste (subproduct) with high generation volumes and undervalued biological potential, as in the case of coffee pulp [5]. Coffee pulp is reported to be used in compost, animal feed, biofuel, and to obtain chemical products; however, the lack of a sustainable alternative means its use is still limited [6].

Procyanidins have hydroxyl groups, chiral carbons, and interflavanic bonds that make them reactive molecules; that is, they can form bonds with other molecules such as proteins and carbohydrates. These compounds can inhibit the growth of fungi and bacteria. However, there are filamentous fungi that are not inhibited [7–9].

A. niger strains are generally recognized as safe (GRAS) and can use PCs as a carbon source to produce enzymes involved in degradation and/or biotransformation processes [10,11]. Degradation refers to the complete loss of a compound (PC) while biotransformation indicates the modification of a compound. This occurs by enzymatic action or a chemical agent, where procyanidins react with other molecules, allowing for the formation of new molecules with differences in their structure and properties [12].

Microbial degradation of tannins or polymeric procyanidins could become a key solution for the valorization of coffee pulp, as well as an alternative method to degrade macromolecules into structurally less complex and toxic compounds, and could contribute to the removal and clean-up of ecosystems [13].

Few studies have considered the degradation of PCs from plant sources of high risk to human health and the environment. There are works that report on the extraction and purification of PCs for degradation studies [14,15]. Contreras et al. [16] investigated procyanidin B2 (PB2) degradation using extracellular extracts from submerged fermentation with *A. fumigatus*, a pathogenic strain previously isolated from coffee pulp. The results suggested the degradation of PB2 dimer by the action of an oxygenase enzyme. Roopesh et al. [11] studied the biotransformation of apple procyanidins by solid-state fermentation with *A. fumigatus*. A biotransformation study of PB2 (an m/z of 577) was performed on the fermentation extracts and the purified enzyme. HPLC-MS allows for the visualization of several biotransformed products, including PB2-X, PB2-X2, PB2-X3 with an m/z of 609, 641 and 609, respectively. These authors attribute these structural modifications in the PB2 molecule to the oxidation of the catechol ring by the dioxygenase enzyme.

On the other hand, due to the multiple advantages of solid-state fermentation, PC characterization and biotransformation studies were conducted by Wong-Paz et al. [12,17]. They reported the presence of PC oligomers in coffee pulp residue and studied the biotransformation of procyanidins extracted from coffee pulp, which were used as a carbon source for the growth of *A. fumigatus* in solid-state fermentations. Fermentation extracts were obtained from the bioprocesses, which were used as an enzymatic source to degrade procyanidin C1 trimer (PC1) in a model system. The authors reported seven biotransformed products, named PC1-X1, PC1-X2, PC1-X3 (m/z of 897), PC1-X4 (m/z of 897), PC1-X5 (m/z of 929), PC2-X6 (m/z of 929), and PB2 (m/z of 577).

Despite these preliminary advances, no studies have reported on the degradation of PCs to monomeric compounds. Therefore, our research group proposed a new design for the fermentation bioprocess, which consisted in the study of PC1 degradation or biotransformation using extracellular extracts from submerged fermentation with a species of *A. niger* (GRAS), where PCs were extracted from coffee pulp and subsequently added to the fermentation medium in a delayed manner. Thus, the evaluation of new bioprocess conditions could favor the growth of *A. niger* and its ability to degrade PCs into less complex compounds. In this study, the biodegradation of procyanidins was evaluated in a model system using PC1 as a substrate and extracellular extracts from submerged fermentation with *A. niger* GH1.

2. Materials and Methods

2.1. Materials

Coffee pulp was harvested from coffee plantations (*Coffea arabica*) in Xilitla, S.L.P., and transported to the Food Laboratory of the Universidad Autónoma de San Luis Potosí, Ciudad Valles, México. The coffee pulp was sun-dried for 72 h and stored at room temperature until use. All chemical reagents were of analytical and chromatographic grade. Acetic acid, acetonitrile, and methanol were purchased from J.T. Baker (8027 Forsyth Boulevard, St. Louis , MO 63105, USA). PC1 was purchased from Sigma Aldrich (St. Louis, MO, USA).

2.2. Extraction and Purification of Procyanidins

The coffee pulp (80 g) was mixed with 800 mL of aqueous acetone (70% v/v). Subsequently, the mixtures were placed in an ultrasonic bath (CPX 3800, Branson, MO, USA) (40 khz) for 10 min. The samples were filtered under a vacuum (V-100, Butchi, Flawil, Switzerland) and the supernatant obtained was concentrated by rotoevaporation (R-100, Butchi, Switzerland). Finally, the crude extract was stored at 4 °C until analysis. The concentrated samples were applied in separatory funnels filled with Sephadex LH-20, previously equilibrated with water. The samples were eluted with water, ethanol, and acetone (70% v/v). The aqueous fraction of acetone containing the PCs was collected manually, rotaevaporated, and oven-dried at 40 °C.

2.3. Microorganism

Aspergillus niger GH1 was selected for this study according to results reported by Valencia-Hernández et al. (2021). This strain was isolated from the Coahuila desert *Larrea tridentata* leaf by Cruz-Hernández et al. [18] and provided by the Department of Food Research, Universidad Autónoma de Coahuila (Saltillo, México). The microorganism was maintained on a potato dextrose agar (Sigma, Kawasaki, Japan) and incubated at 30 °C for 8 days.

2.4. Growth Medium

The spores (8 days old) were suspended in 50 mL of sterilized distilled water containing 0.01% (v/v) Tween 80 and used as inoculum for submerged fermentation. The initial fermentation medium contained the following (g/L): NaNO3, 6.0; KCl, 0.5; KH2PO4, 1.5; $MgSO_4 \cdot 7H_2O$, 0.5; $FeCl_3$, 0.0085; $ZnSO_4$, 0.001; $C_6H_{12}O_6$, 10 g/L. Additionally, 1 mL of oligo-element solution contained the following (g/L): $Na_2B_4O_7 \cdot 10H_2O$, 0.1; $Na_2MoO_4 \cdot 2H_2O$, 0.05; $MnCl_2 \cdot 4H_2O$, 0.05; and $CuSO_4 \cdot 5H_2O$, 0.25. The medium was supplemented with 1 g/L of a PC solution as a secondary carbon source. PCs were added to the culture medium once the glucose was consumed by the microorganism to promote its growth. The pH of the fermentation was adjusted to 6 using NaOH (1.0 M).

2.5. Fermentation Conditions

Submerged fermentation was carried out as described by Valencia-Hernández et al. [9]. A total of 400 mL of medium was inoculated with *A. niger* GH1 (5×10^7 spores per gram of carbon source) and dispensed into amber reactors at a final volume of 10 mL. The reactors were incubated at 30 °C with a shaking speed of 120 rpm. For kinetic study, samples were taken after 0, 12, 24, 24, 24, 48, 48, 72, 96, 120, 144 and 196 h. For each sample, 3 amber reactors were taken, where the fermentation broth was manually separated from the mycelium. The controls consisted of reactors containing the fermentation broth without PCs.

The fermented broth containing PCs was subjected to acid hydrolysis and the content of these compounds was determined by the HCl–butanol method described by Porter et al. [19]. Total sugars and reducing sugars were determined as described by

Miller [20]. For biomass determination, the mycelium was oven-dried at 75 °C to a constant weight. Biomass and sugars were expressed in g/L and procyanidins in mg/L.

2.6. Extracellular Extract

The enzyme extract was obtained from fermentation kinetics performed with *A. niger* GH1. The extracts were collected once the PCs were added to the fermentation medium, then filtered with a syringe filter (PTFE, 0.45 µm, Millipore, Naucalpan de Juárez, Mexico) and stored at 4 °C until use.

2.7. Enzyme Assay in a PC1 Model System

The PCs' degradation potential of the enzyme collected from three fermentation extracts was determined according to Wong-Paz et al. [12]. The enzyme assay was performed by mixing 50 µL of each extract with 200 µL of PC1 trimer solution reconstituted in a phosphate buffer, with a pH of 6 (0.2 M). The final concentration of the PC1 solution was 577 µM. The reaction was carried out at 30 °C for 2 h. One unit (U) of activity was expressed as the amount of enzyme required to degrade 1 µ mol of PC1 trimer per minute.

The extract showing the highest enzymatic activity was selected to carry out kinetic testing at the following specified times: 0, 2, 4, 6, 8, 10, 12, and 24 h. The reaction mixtures were similar to those described above, increasing the final concentration of the PC1 trimer solution to 1000 µM. Identification of PC1 degradation products was carried out by HPLC-MS at 280 nm.

2.8. Analysis of the Biotransformed Products of PC1

The PC content and biotransformed products obtained after the enzyme assay using PC1 were characterized by a high-performance liquid chromatography (HPLC) system coupled to an ion trap mass spectrometer (Varian 500-MS IT Mass Spectrometer, Varian Inc, Palo Alto, CA, USA) with an electrospray ion source (ESI-MS). The system was equipped with an autosampler (Varian ProStar 410, Walnut Creek, CA, USA), a ternary pump (Varian ProStar 230I, Palo Alto, CA, USA), a PDA detector (Varian ProStar 330, Palo Alto, CA, USA), and a Denali C18 column (150 mm × 2.1 mm, 3 µm, Grace, Columbia, MD, USA). Samples (5 µL) were injected into the C18 column. The oven temperature during analysis was maintained at 30 °C. The mobile phase was a mixture of formic acid (0.2%, *v*/*v*; solvent A) and acetonitrile (solvent B). The following gradient elution profile was used: initial, 3% B; 0–5 min, 9% B linear; 5–15 min, 16% B linear; 15–45 min, 50% B linear. A PC1 standard curve was performed to calculate the content of PCs present in the extracellular extracts. The column was then washed and reconditioned. The flow rate was 0.2 mL/min and elution was monitored at 280 nm. The whole effluent (0.2 mL/min) was injected into the source of the mass spectrometer without splitting. MS analysis was conducted in negative ion mode $[M\text{-}H]^{-1}$. Nitrogen was used as a nebulizing gas and helium as a damping gas. The ion source parameters were a spray voltage of 5.0 kV, temperature of 350 °C, and capillary voltage of 90.0 V. Data were collected and processed using MS Workstation software (Version 6.9).

3. Results and Discussion

A. niger GH1 was selected because it degraded procyanidins until the end of fermentation. Due to the scope of the project, this research only describes the degradation of procyanidins in *A. niger* GH1. Enzymatic assays were carried out in the following two steps: First, three extracellular extracts from the fermentation times with the highest procyanidin degradation, measured by the HCl–butanol method, were evaluated (obtained in stage 2). Second, the extract with the most increased enzymatic activity was selected (Figure 1) and

an enzymatic kinetic assay was performed to determine PC1 degradation with the selected extract (Figure 2).

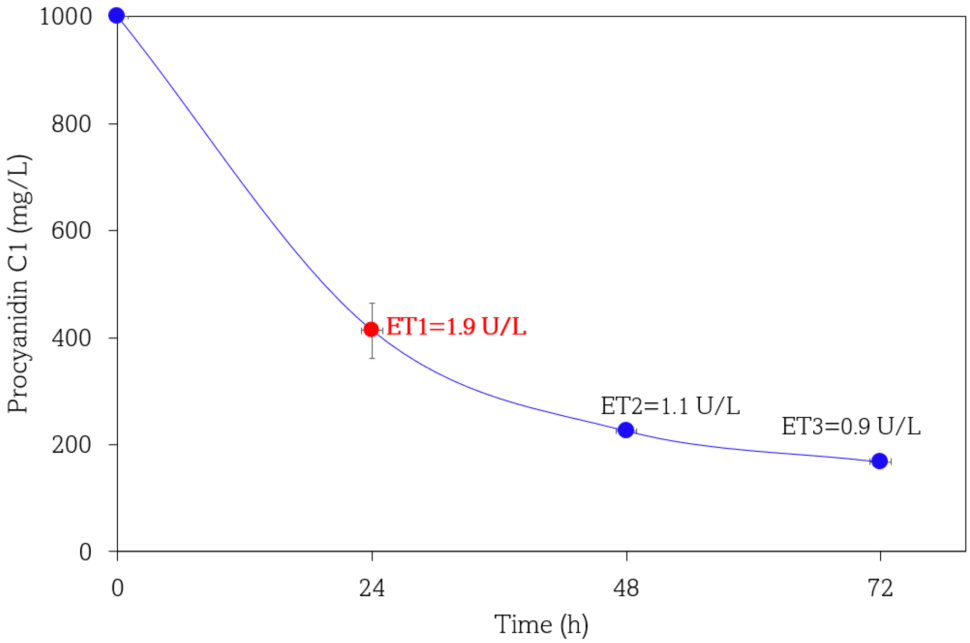

Figure 1. Procyanidin (PC) removal and enzymatic activity of the extracts (ET1, ET2, and ET3) in submerged fermentation with *Aspergillus niger* GH1 (577 µM), calculated by the HPLC-MS method.

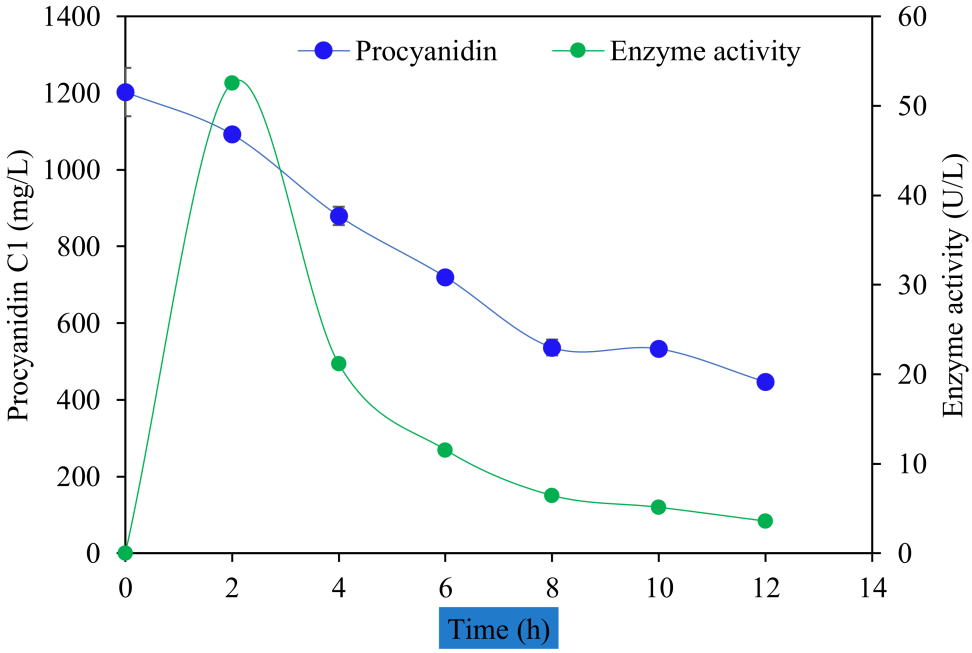

Figure 2. Procyanidins (PC) removal and enzymatic activity in submerged fermentation with *A. niger* GH1 (1000 uM), calculated by HPLC-MS method.

Figure 1 shows the enzymatic activity of the fermentation extracts, where a decreasing behavior was observed. The maximum enzymatic activity recorded was in the extract obtained at 24 h of fermentation (1.9 U/L), which coincides with the highest degradation of procyanidins for this fungus (the data are shown in stage 2). Subsequently, the possible PC-degrading enzymatic activity decreased in the extracts obtained at 48 and 72 h. Therefore, the extract obtained at 24 h of fermentation with *A. niger* GH1 was selected as the enzyme source to degrade procyanidins in a model assay with commercial PC1 at different reaction times.

Investigations have indicated the influence of nutrients in the culture medium on enzymatic activity. Hossain et al. [21] observed the effect of different culture media on the enzymatic activity of *A. niger*, with the Czapek–Dox medium having the highest activity. Thus, it is more attractive for enzyme production. Using maltose as a substrate, Asrat and Girma [22] produced a maximum alpha-amylase activity of 0.684 U/mL in *A. niger* FAB-211. Shoaa-Muzaffar et al. [23] obtained a laccase activity of 42.91 U in *A. niger* after 48 h of fermentation using a medium containing glucose, potassium, magnesium, calcium and urea. Maximum enzymatic activity occurred at maximum glucose utilization, which is similar to this study, where the highest enzymatic activity was obtained at the fermentation time where there was a greater degradation of procyanidins. Roopesh et al. [11] and Contreras-Dominguez et al. [16] were the first to report evidence of the fungal degradation of procyanidins using a strain of *Aspergillus fumigatus*. They identified a novel aromatic ring-cleavage product, confirming a lactone formation in epicatechin moiety involved in oxidative degradation, and suggested the action of a dioxygenase enzyme was responsible for the degradation.

The results obtained show the efficacy of procyanidins as an energy source, even in the absence of glucose. Furthermore, although specific concentrations of these compounds can be inhibitory to fungal growth, they provide satisfactory enzymatic activity. However, we recommend trying to supplement the culture medium with a nitrogen source, which has been reported to increase the level of certain enzymatic activities. This fact could enhance the degradation of procyanidins. *A. niger* GH1 grew on a basic salt medium containing glucose as a primary carbon source and procyanidins as a secondary source once the glucose was consumed in its growth phase. This experiment aimed to initially increase the growth and metabolic activity of the microorganism, as scientific contributions indicate that carbohydrates are factors that affect the metabolism of the fungus. In some cases, it could decrease the production of some enzymes. In this study, glucose was used to stimulate hyphal growth in mycorrhizal fungus, after which the production of procyanidin-degrading enzymes was stimulated by adding a second carbon source, which has been reported in previous studies as an inducible substrate to produce procyanidin-degrading enzymes [11,16].

Procyanidin removal and the production of the degradative enzyme present in the filtered culture of *A. niger* GH1 are presented in Figure 2. These results show a decrease in behavior in the concentration of procyanidins over 12 h of incubation, due to possible degradation. These results are also consistent with those obtained in step 2 for procyanidins measured by the HCl–butanol technique. The enzymatic activity also showed a decrease, where the maximum value was obtained after 2 h of incubation, then gradually decreased as the procyanidin concentration decreased. The highest enzymatic activity was reached after 2 h of incubation; however, the procyanidin removal was higher than that obtained in this study. These findings indicate that enzymatic activity is related to the fungal species and procyanidin concentration in the enzyme assay. Moreover, the quality of the substrate, reaction time, and reaction pH are also relevant factors that determine the rate of degradation. Maximum enzymatic activity is reached at a certain pH, which is called optimum [24]. Khan and Murphy [25] reported the capacity of *Cunninghamella elegans* to biotransform xenobiotic compounds, considering cytochrome P450 (CYP) can be involved in the metabolic reaction. In addition, the authors mention that CYPs could catalyze monoxygenation reactions, which have been reported in the degradation of tannins.

According to what has been reviewed, no scientific studies have reported on this subject. *A. niger* acts within a wide pH range (1.5–9.8) [26,27] and this fungus can use monocyclic aromatic compounds as its only source of energy and carbon. Researchers have reported the evaluation of pH on the activity of different enzymes. In previous studies,

the characterization of dioxygenase enzymes responsible for the degradation of aromatic compounds of *Aspergillus* species has been reported [11,28,29]. However, other studies, such as that of Hu et al. [30], obtained an optimal pH between 5.5 and 6.5 for enzyme production. Ire et al. [31] also obtained maximum xylanase activities at a pH of 5.0 using corn cobs and sawdust as substrates.

HPLC-ESI-MS analysis allowed for the monitoring of the biotransformed products of a model solution of PC1. PC1 was reacted with the enzyme extract of *A. niger* GH1 at 1000 µM during a 1440 min reaction, followed by HPLC-ESI-MS. In Figure 3, it can be observed that the procyanidin C peak accompanies the catechin peak. The chromatogram (a) shows a similar profile to that obtained after the enzymatic reaction (b), where a small reduction in the procyanidin C peak is observed, along with the appearance of new peaks, including a possible compound, catechin. Chromatogram (b) differs from (a) due to the appearance of new peaks, which we suggest are the result of the degradation of the initial compound. However, PC1 showed little variation in peak intensity, which may be related to limited degradation. The minimal degradation of procyanidin was not investigated but is a topic for further study.

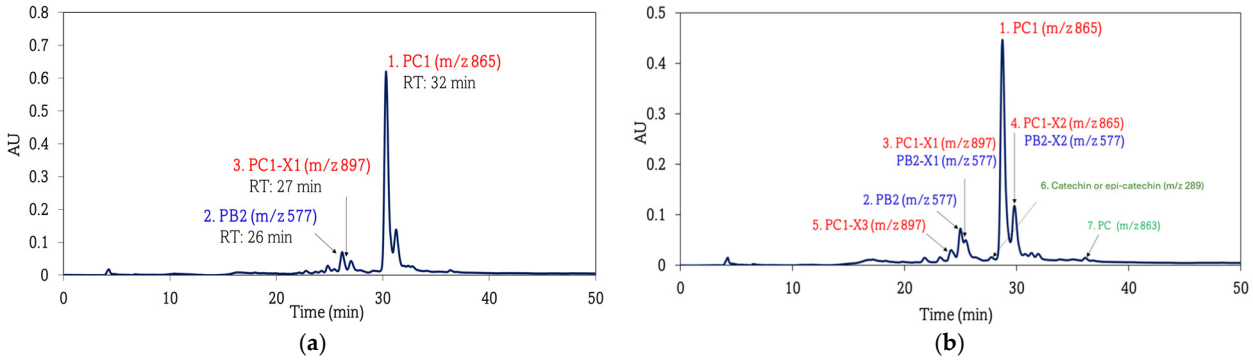

Figure 3. Chromatogram (HPLC-MS): (**a**) procyanidin C1 (PC1) and its (**b**) degradation products during 12 h of enzymatic reaction using extracellular extract from submerged fermentation with *A. niger* GH1.

A typical HPLC profile of the PC1 standard (peak 1) at the start of the enzyme assay is shown in Figure 3. After 120 min of enzymatic reaction, two peaks appeared. Peak 2 appeared with an RT of around 26 min and remained unchanged during the assay, followed by peak 3 (RT 27 min). After 480 min of incubation, another compound, with an m/z of 577, appeared in peak 3. Peak 3 remained until the end of the incubation. Compounds with the same m/z were observed in peaks 2 and 3; therefore, they were named PB2 and PB2-X1. The m/z of 577 corresponded to a decrease of 289 atomic mass units compared to the m/z of the PC1 molecular ion. This decrease may be due to the loss of a monomeric unit by enzymatic action. In this case, it could be suggested that PC1 may be degraded to PB2 by dioxygenase enzymatic action, documented by Wong-Paz [32], who observed a similar decrease in a biotransformation study of PC1 in a solid medium using an enzymatic extract from fermentation with *A. fumigatus*.

The second degradation product (peak 3) was named PC1-X1, with an m/z of 897, corresponding to an increase of 32 atomic mass units compared to the molecular ion of PC1. This peak is a product of the biotransformation of PC1, where the catechol ring is oxidized and the molecule subsequently converted into a non-phenolic residue by the enzymatic action of dioxygenase. In addition, they obtained a compound with the same m/z, but the MS/MS assays confirm that these compounds are isomers. According to the author, the retention times also indicate that they are molecules of equal molecular weight but differ in their polarity. In our study, this compound showed no similarity to other compounds

in m/z. To our knowledge, ours is the second study where PC1-X1 was identified as a degradation product of PC1.

Peak 4 appeared after 240 min of enzymatic reaction; the compound eluted at a retention time of 33 min with an m/z of 865. After 480 min, a new compound was eluted in peak 4, corresponding to a compound with an m/z of 577, possibly corresponding to PB2. These compounds appear in the chromatogram (Figure 3) as PC1-X2 and PB2-X2. The mass spectrum of compound PB2-X1 was similar to PB2-X2, with a shorter RT, indicating its polarity is higher. In a model biotransformation study with PB2, Roopesh et al. [11] reported similarly charged masses in degradation studies of a commercial procyanidin. As reported in the literature, procyanidins have asymmetric carbons and interfacial bonds that allow the molecule to form stereoisomers, dimers, trimers, and tetramers. Toro-Uribe et al. [33] obtained procyanidins from cocoa polyphenolic extract, finding a mixture of chemical structures similar in m/z, which were closely eluted without allowing for the complete separation of the oligomers. The authors indicate that the cleavage of the catechol ring occurs in the PC1 extension unit. The formation of the PB2-X2 molecule could occur from the elimination of a monomeric unit (m/z of 289) in PC1-X2.

Peaks 5 and 8 presented an m/z of 897 and 865, respectively. Peak 5 appeared after 480 min with an RT of 25 min and remained unchanged until the end of the assay. This peak was namedPC1-X3. Peak 8 was identified as a possible isomer of PC1, named PC1-X3, which appeared after 720 min at an RT of 32 min. PC1-X3 was considered an isomer of PC1-X1. Based on the retention time of the catechin and epicatechin standards, it is suggested that these compounds may be present in the enzymatic extract (peak 6) with an m/z of 289 and a retention time (RT) of 29 min. Moreover, previous papers describe the presence of catechin and (epi)catechin via ions at an m/z of 289 and B-type procyanidins at an m/z of 577 (Wang et al. [34]) at a UV wavelength of 280 nm. In the HPLC-ESI, catechin and epicatechin were detected at an m/z of 289 (Yuzuak et al.) [35]. From chromatograms of berry extracts, Cangeloni et al. [36] reported the m/z ratios for (+)-catechin and (−)-epicatechin as 289.1574 and 289.1586, respectively. On the other hand, our research group is interested in studying the MS2 fragmentation of procyanidin monomers. It is known that the diastereomers of catechin and epicatechin have been observed at an m/z of 203, 205, and 245 in MS2 fragmentation (Silva et al.) [37]. However, important data are still needed to help us elucidate the biological degradation mechanism of these types of compounds.

Peak 7 could correspond to a procyanidin type A trimer with an m/z of 863 and eluted at an RT of 37 min. Previously, the latter compound was identified by HPLC-ESI-MS in the acetone fraction of coffee pulp extracts and subsequently analyzed by MS/MS, confirming the presence of a procyanidin oligomer. Both bioproducts of the degradation and biodegradation of PC1 are shown in Figure 3. The compound with an m/z of 289 was detected after 480 min of enzymatic activity, where it could be hypothesized to be the final product of the degradation or biotransformation of PC1. So far, no monomeric units of PCs have been reported in the literature in fermentation degradation studies using *Aspergillus* strains.

The antioxidant power of polyphenols is well-known, as well as their importance in the prevention of diseases with high mortality rates in humans, among the most reported being cancer, neurodegenerative disease, and cardiovascular disease (ReFaey et al. [38]. Due to this, the preference for a healthy lifestyle and the consumption of functional foods or dietary supplements has increased in the population. Studies have discussed the biological properties of catechins, epicatechins, and procyanidin types A and B in the treatment of cardiovascular, neurodegenerative, and autoimmune diseases. Furthermore, reports indicate the benefits procyanidins incorporated into food provide for intestinal health. Carriere et al. [39] reported that an A-type PA trimer (Cinnamon B-1) is related to the

expression of antiapoptotic proteins, inhibiting colon cancer cells such as DLD-1 and COLO 201. Catechin inhibits processes of proliferation and migration of cancer cells related to the PI3K/Akt pathway (Ding et al.) [40]. Epicatechin, an isomer of catechin, has been mentioned as an inhibitor of tumor progression and in the prevention of inflammatory diseases. Thomas and Dong [41] demonstrated its effectiveness in inducing apoptosis in prostate and breast cancer cells such as ZIP9.

Other enzymes, such as laccase, peroxidase, dioxygenase, and cytochrome P450, have been involved in the biodegradation/biotransformation of these types of chemical compounds. For example, Khan et al. [42] described the key enzymes involved in xenobiotic biotransformation and the potential of fungal strains and their enzymes in the bioremediation of polluted environments. Dave and Das [43] reported information on microbial enzymes from various microorganisms and mechanisms for the biodegradation of an extensive range of pollutants, in particular, laccases and hydrolases.

To our knowledge, this is the first report of PC degradation to single molecules. The study that comes closest to the results reported here is the one reported by Contreras-Domínguez et al. [13]. In their study of PC degradation by *A. fumigatus*, the authors observed a peak with an m/z of 289 when PB2 was subjected to a thiolytic reaction. This technique allowed for the characterization and quantification of monomeric procyanidin units, which could be applied to future PC degradation studies with *A. niger* GH1. Finally, after 720 min of incubation, no other compounds were observed. Studies have indicated that *Aspergillus* spp. can produce lignocellulosic degrading enzymes of lignocellulosic components and mono- and polynuclear aromatic pollutants. All these compounds are characterized by the fact that they are difficult to degrade in the environment [44–46]. The degradation or biotransformation of aromatic compounds has been reported in a variety of microorganisms. The biodegradation phenomenon occurs through metabolic pathways that can be classified as convergent and divergent, where aromatic compounds can be degraded to catechol, protocatechuate, and their derivatives, which can be intermediates of other pathways. In divergent cases, these include enzymes such as metal-dependent dioxygenase channel intermediates [47].

PC1 removal during the enzymatic assay agrees with the appearance of new compounds with higher and lower masses. The HPLC-MS results clearly demonstrate the degradation and biotransformation of PC1 by enzymes produced by *A. niger* GH1 during submerged fermentation. The results of the enzymatic activities suggest that *A. niger* GH1 could be considered a producer of procyanidin-degrading enzymes and a possible degrading microorganism under submerged fermentation conditions. However, we consider that further research should be conducted on the ability of *A. niger* GH1 to produce these enzymes, investigate the optimization of the process parameters to produce these enzymes, as well as those in other fungal strains with a procyanidin-degrading ability.

Researchers are exploring new sources of antioxidant phytochemicals such as procyanidins. Among them, Chen et al. [48] have demonstrated the biological potential of coffee phytochemicals in terms of their properties, such as antioxidant, antibacterial, anti-inflammatory, antihypertensive, etc. Documentation on the extraction of these compounds has increased in recent years, as new insight is needed for the development of applications relevant to human health. The high generation of value-added products in the industrial sector has benefited from enzymatic strategies that improve the nutritional, functional, and sensory properties of food products. However, as reported by Weng et al., the food industry faces challenges in finding alternatives to protect enzymatic activity, as this characteristic can be reduced under certain adverse conditions of temperature and pH [49]. Additionally, enzyme production presents limitations for large-scale manufacturing and environmen-

tal sustainability. As one alternative, Zheng et al. propose the use of high-performance biocatalysts which could allow for industrialization [50].

4. Conclusions

The *A. niger* GH1 strain showed potential for degrading procyanidins from coffee pulp. The compounds identified by HPLC-MS confirmed the degradation of procyanidin C1 under the assay conditions, which could be used to understand biodegradation pathways proposed for future study. The extracellular extract of *A. niger* GH1 was able to degrade PC1 into monomeric molecules. To our knowledge, such products have not been reported in the literature under the fermentation and enzymatic assay conditions described. Therefore, our study provides the first report of the degradation of PC1 using enzymatic extracts from submerged fermentation with *A. niger* GH1.

These results indicate that tannin-degrading fungi are not only promising for the production of bioactive compounds, but also have potential in the management and handling of agricultural waste, in this case, coffee pulp. Microorganisms such as the *Aspergillus* sp. could be used in detoxification processes of plant material, in pretreatments for fermentation processes, and can assist in the removal of toxic agents from liquid effluents. However, further study of the degradation pathway of these filamentous fungi is necessary to explore new applications in the food industry aimed at environmental sustainability.

Author Contributions: Conceptualization, L.J.V.-H. and C.N.A.; methodology, L.J.V.-H., J.A.A.-V., J.E.W.-P., A.P.-B. and C.N.A.; software, L.J.V.-H. and C.N.A.; validation, L.J.V.-H., J.A.A.-V. and J.E.W.-P.; formal analysis, L.J.V.-H.; investigation, L.J.V.-H.; resources, M.L.C.-G., J.E.W.-P. and C.N.A.; data curation, L.J.V.-H., J.A.A.-V., J.E.W.-P. and C.N.A.; writing—original draft preparation, L.J.V.-H.; writing—review and editing, L.J.V.-H., J.A.A.-V., J.E.W.-P., M.L.C.-G. and C.N.A.; visualization, L.J.V.-H. and C.N.A.; supervision, J.A.A.-V. and J.E.W.-P.; project administration, M.L.C.-G. and C.N.A.; funding acquisition, C.N.A. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by CONACYT, grant number FOSEC_CB 2017-18 A1-S-42515. The APC was funded by the project CONACYT-SEP-CB A1-S-42515.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data supporting reported results can be requested to the corresponding author.

Acknowledgments: The authors thank the National Council of Science and Technology (CONACYT, México) for the financial support given to this project through the Basic Science program, CONACYT-SEP 2017-18 CB-A1-S-42515, and the national scholarship program of the PNPC-CONACYT (CVU 1013484).

Conflicts of Interest: The authors declare no conflicts of interest.

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